

**TECHNICAL REPORT**

**IN VITRO AND IN VIVO STUDIES FOR A BIO-IMPEDANCE  
VITAL-SIGN MONITOR**

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## **BACKGROUND**

### *Warfighter Physiological Status Monitoring (WPSM) Research Program*

The WPSM program is a multi-institute research program focused on gathering, archiving, and interpreting physiologic data from warfighters in the field. The ultimate goal is to develop a suite of wearable sensors to provide critical physiologic information to commanders and medics. Biosensors, personal area networking, and data management hardware and software provide a wealth of physiologic information within a broader environmental framework. Data can be used to develop models of thermal stress, hydration status, cognitive state, etc. The WPSM program is progressing in an iterative manner, utilizing the test-model-test methodology.<sup>4-6</sup>

Although there is a designated institution for the “integration of computers and electronics with textiles” there were no presentations on wearable electrodes at the 2001-2005 ATACCC Conferences.<sup>7</sup>

Electrical impedance (bioimpedance) shows promise as a noninvasive method for monitoring the physiological status of soldiers in the Land Warrior and Future Warrior.<sup>8-9</sup>

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There were no conflicts of interest related to this study.

## **EXECUTIVE SUMMARY**

The goal of Army Warfighter Physiological Status Monitoring, a subgroup of Land Warrior program, is to develop a suite of wearable sensors to provide critical physiologic information to commanders and medics.

Bio-impedance can be used for peripheral pulse detection as a non-invasive method for continuous vital sign monitoring.

The objective of this study was to evaluate the commercially available electrode materials that might be useful as wearable electrodes for the measurement of bio-impedance pulse wave, to measure pulse variability, and test pulse detection sensitivity.

Electrode down-selection was performed based on in vitro and in vivo studies. A total of 13 conductive materials and 13 subjects were measured. Arm cuff inflation was used to measure pulse sensitivity. Two PCs were used for data collection.

Reproducibility and sensitivity of the bio-impedance measurement were comparable to the sensitivities of the pulse oximeter, laser Doppler, and Doppler ultrasound. There was no statistical difference between the bio-impedance measurement and the other techniques.

Results demonstrated that bio-impedance offers potential for use as a multifunctional, continuous, non-invasive life sign monitor for both military and civilian purposes.

## INTRODUCTION

The two most frequent causes of death in combat are exsanguination (44%) and central nervous system injury.<sup>1</sup> Development of devices for early noninvasive monitoring of multiple parameters in the field is indicated.<sup>2</sup> An important goal of the Army's Medical Department is to develop physiological monitoring of parameters that will aid in the assessment and treatment decisions of combat casualties in order to decrease combat mortality. The potential time frame of combat casualty care (CCC) – window of therapeutic opportunity - was described earlier.<sup>1-3</sup>

### *Bioimpedance*

Electrical impedance instrumentation is relatively low cost, which has encouraged its possible application in many different areas. The impedance measurement is influenced by many different factors, including geometry, tissue conductivity, and blood flow. Because of this complexity, it is difficult to measure reliably an isolated physiologic parameter, the principal factor limiting its use. The applications widely used in clinical medicine are apnea monitoring and the detection of venous thrombosis. The other applications described will need more study before becoming reliable and useful measurements.<sup>10</sup>

To build a bioimpedance life sign monitor, it is necessary to select an electrode material that can be sewn into a uniform. Then, the resulting electrode must measure the variability and sensitivity using the bioimpedance pulse wave. This information was not available. Therefore, we evaluated various electrode materials. There are no specific existing FDA guidelines on the topic; however we tried to comply with the guidelines that are both available and closest to performance and quality assurance testing.<sup>11,12</sup> Related literature gives variability studies on other applications but not bioimpedance peripheral pulse.<sup>13-17</sup> Although available industrial products can be used to measure vital signs, they are not appropriate for military applications, because they are invasive and are not suitable for sewing into a conductive fabric,<sup>18</sup>

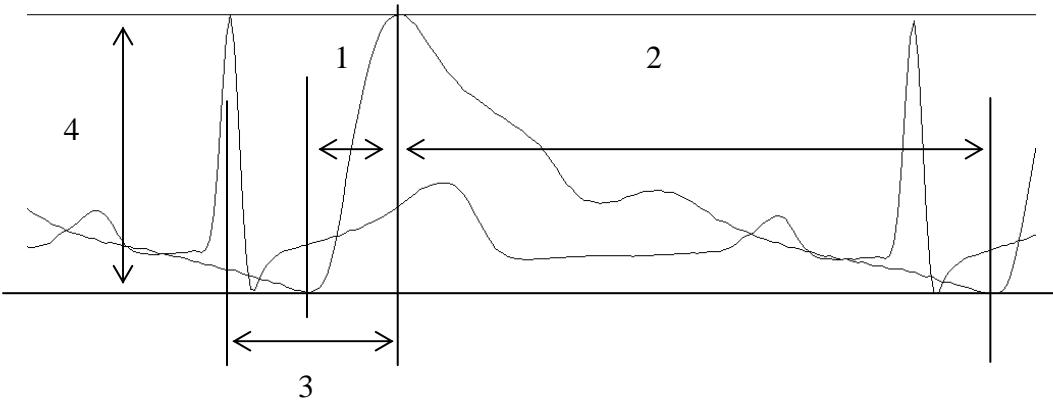
A related question is to determine which clinically used vital signs are applicable for monitoring on the battlefield, a concern of particular importance today because of increased threats of bioterrorism.<sup>20</sup> One main biological/monitoring question to explore is the sequence of cessation of vital signs. The modality providing the earliest indication of a life threatening condition is ideal for monitoring. Although in clinical practice, ECG and pulse oximeter are the methods most widely used, ECG is the last vital sign to disappear during hemorrhage.<sup>19</sup>

### *Technical Aspects*

Electrical impedance offers advantages as a technique for life sign monitoring, especially in field operations. The technique is non-invasive, portable, lightweight, and easy-to-use. In order to make this advantageous method available for use on the battlefield, standards and automated computation procedures must be established. It is known that bio-impedance can be used for non-invasive monitoring of circulation (peripheral pulse).<sup>21,22</sup> This technique also shows potential for monitoring the physiological status of injured soldiers as a non-invasive blood pressure estimation,<sup>23</sup> for monitoring during the transport of wounded soldiers,<sup>24</sup> and for civilian use during emergency treatment.

Conductive elastic fabrics that might be used as wearable electrodes sewn into a uniform have recently become commercially available and may be ideal for incorporation into the new Advanced Combat Uniform and the Scorpion Uniform, under design for the Land Warrior and Objective Force Warrior programs. Until recently, the available conductive materials suitable for wearable electrodes have not been tested nor have the basic characteristics of pulse wave transmission time (PWTT) been identified.

PWTT is a method for acquiring a noninvasive approximation of blood pressure based on the time delay between the R-wave of an ECG signal and the arrival of the arterial pulse at a peripheral site<sup>23</sup> (Fig. 1). The Cerberus system was designed for cardiovascular screening, using six channel bio-impedance measurements calculating the amplitude maximum of pulse waves and PWTT.<sup>25,26</sup> With the used Cerberus system, the ECG signal is synthesized from impedance electrodes. This feature makes it possible to use conductive fabrics as multifunctional sensors.



**Fig. 1.** Bioimpedance/REG pulse curve and its relationship to Electrocardiogram (ECG) and basic variables of REG. The pulse wave transmission time is defined as the time between the peak of the R-wave of the ECG signal and the peak of the bioimpedance pulse. Peak time is the time from the start of the pulse until its peak, called anacrotic time. The descending pulse wave is called catacrotic time. Pulse amplitude is the difference between the minimum and the maximum value of a pulse. 1: anacrotic part (81.1 ms), 2: catacrotic part, 3: pulse wave transmission time (152 ms), 4: pulse amplitude, Period:  $1 + 2 = \text{ECG R} - \text{R interval}$ : 618.4 ms. Typical healthy REG curve (Cerberus record ID# 1). Fragment of original trace; time window 934.5 ms (X axis is ms, Y axis arbitrary unit – units of AD conversion) ECG trace also presented.

## OBJECTIVE

The objectives of this study were

- 1) to evaluate the commercially available electrode materials that might be useful for the measurement of bio-impedance pulse wave,
- 2) to measure pulse variability, and

3) to test pulse detection sensitivity.

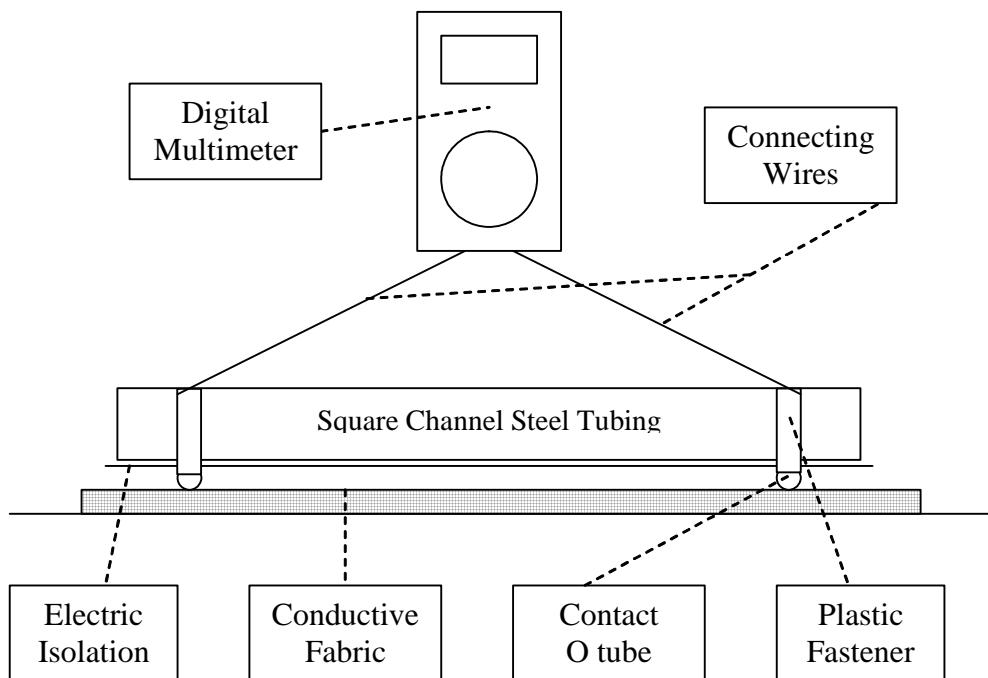
## METHODS

The electrode down-selection was performed based on *in vitro* and *in vivo* studies. A total of 13 conductive materials and 13 male subjects ( $35.92 \text{ y} \pm 13.78$ ) were measured.

During down-selection, 13 conductive materials were selected for *in vitro* testing of the electronic conductivity and potential applicability as a peripheral electrode for bio-impedance. From these 13 materials and 5 conductive fabrics (personal antistatic grounding) were chosen for *in vivo* tests in the first phase; in the second phase, the two best fabrics were identified.

### *In vitro*

The goal of this phase of work was to find the best electrode that could be sewn into a uniform to monitor physiological status. To compare electrical resistance of 13 conductive materials, a measuring arrangement was created.



**Fig 2.** Block schematics of the in vitro test. The conductive fabric was placed on the desk under the square channel steel tubing holding the two electrical contacts connected to a digital multimeter (HD 100, Beckman, Fullerton, CA). The distance of contact O tubes was 10 cm. The fixation of 10 cm was granted by plastic fastener - multi purpose tie (Thomas & Betts Corp, Memphis, TN). The O tube was isolated from the carrier with nylon foil (electric isolation). The connecting wire was soldered to the contact O tube. Ten resistance measurements were performed with altered locations. The data were averaged and expressed as mean  $\pm$  SD, presented in Table 1.

Each conductive material was placed on a flat, nonconductive surface. To secure the electrodes to the conductive materials, a contact carrier was made from an iron metal rod (38 x 38 mm cross section and 23 cm long, weighing 638 gram, including 2 wires to the multimeter, and 2 banana plugs) isolated from the electrical contact and holding two sensors 10 cm apart. The contact sensor consisted of 2 stainless steel tubes with a length of 37 mm and diameter of 5 mm, soldered to a copper wire and banana plug. Ten resistance measurements were made in ten separate locations. The measuring tool was a digital multimeter (HD 100, Beckman, Fullerton, CA), (Fig. 2). The tested materials were as follows: Foster Miller Conductive Fabric, Copper shielding textile, Velcro HI-MEG, Velcro Stainless Steel Loop, Velcro Stainless Steel Hook, Semitronics PK 2050, Rheoscreen (Medis GmbH), SP1201\*, 3M4720\*, 3M2368\*, Bam EA\*, S 7920\*, S 7933\* (\*:All-Spec Industries, Inc., Wilmington, NC).

#### *In vivo General Procedure*

General measurements were performed as follows: a 9-electrode cap (5 scalp EEG + 4 rheoencephalogram -- REG electrodes, Electro-Cap International, Inc. Eaton, OH) was placed on the head of each subject in a sitting position and filled with electrolyte gel (Nicolet Biomedical, Madison, WI). Electrode locations within the cap were as follows: (1) EEG (T5-O1 and T6-O2) recorded in bipolar configuration. EEG ground was Fz; (2) REG (Fp1-F7 and Fp2-F8) in bipolar derivation. Localization is given according to the International 10-20 system of EEG.<sup>27</sup> Subjects were asked to lie in a supine position. For each subject, the conductive fabric was placed around both wrists and ankles; inter-electrode distance was 5 cm. The electrode cap and the peripheral electrodes were

connected to Cerberus system. The Dinamap Pro1000 (GE Medical Systems, Milwaukee, WI) was used to obtain blood pressure and heart rate measurements prior to the testing of each conductive fabric; 10 blood pressure recordings were made. Blood pressure and its derivatives, ECG, EEG, and REG were also measured as a potential source of biological variability. Five 1-minute recording were acquired. After the fifth recording, the conductive fabric was changed, and blood pressure measurements and recordings were repeated for each fabric. In vivo data are presented below in subgroups addressing specific items of interest for this study.

#### *Cuff Pressure Test and Down Selection*

Adult-size Dura-Cuffs (GE Medical Systems, Milwaukee, WI) were placed on two subjects around each of the peripheral electrode arrays. These cuffs applied a pressure of 0, 7.5, 15, and 22.5 hPa (0-3.75 mmHg) on the conductive fabric to ensure that contact resistance would not be a source of variability. The cuff pressure was measured by digital manometer (Testo 505-P2, Flanders, NJ). Five 1-minute recordings were acquired. After the fifth recordings, the conductive fabric was changed, and the blood pressure was measured and recordings were repeated until each of the five fabrics had been tested. A total of 10 measurements for each conductive fabric were recorded at each location (both arms and legs). Down selection was also performed on the two subjects: 5 conductive fabrics were used in five measurements (Hi-Meg, Velcro Industries B.V, Manchester, NH; SP7933, TM2272, TM2204-3M, and SP776 conductor wrist straps, All-Spec Industries, Inc., Wilmington, NC).

#### *Signal Variability*

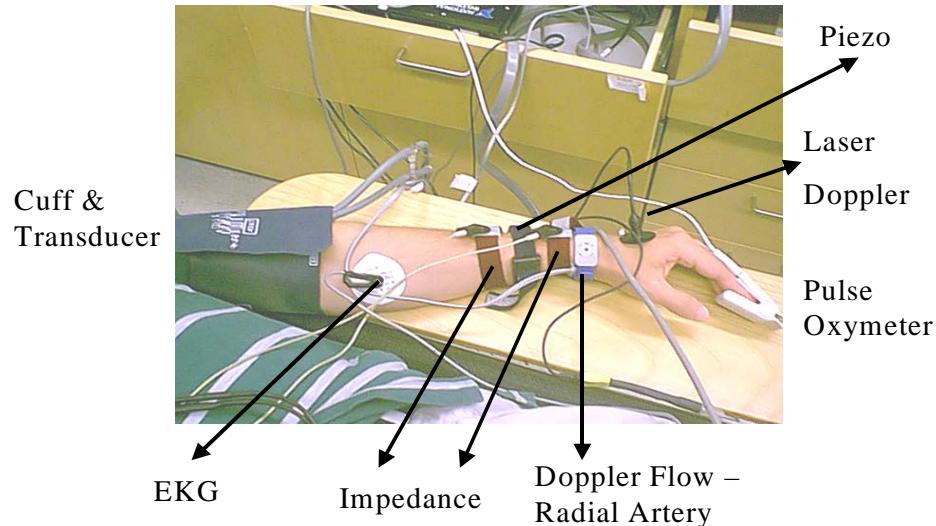
To evaluate reproducibility of a bioimpedance pulse wave, its variability was calculated. Measurements with 2 selected conductive fabrics (Electrode 4, TM2204 and Electrode 5, SP776) were performed on 11 subjects placed in the supine position, with a 1 minute recording time at rest; 5 measurements were made for each conductive fabric using the Cerberus system and another PC to collect multichannel physiological signals (Fig. 3). As

a reference, ECG, EEG and blood pressure variability were used. The main characteristics of the bioimpedance/REG signal are shown on Fig. 1.

### *Pulse Sensitivity*

Next, 1-minute measurements were made with the same two selected conductive fabrics on the 11 subjects with one upper arm cuff placed on the dominant hand. Cuff inflation measurements were made as the pressure on the dominant arm was increased to approximately 200 mmHg, then decreased to 0 mmHg, using Dinamap Pro 1000. Five measurements were made for each conductive fabric. Five of the recordings were control measurements taken before each cuff inflation measurement. Control recordings measured the pulses without any change in pressure on the arm. Changing pressure on the arm allowed the sensitivity of each of the modalities to be analyzed. Bio-impedance pulses were compared to the following signals: a pulse oximeter (Universal Hinged Sensor, Palco Labs, Santa Cruz, CA); a laser Doppler flowmeter with integrating probe (Periflux System 4001, Perimed Sweden); an ultrasound Doppler (Model 811-B Doppler Ultrasonic Flow Detector, Parks Medical Electronics, Inc., Aloha, OR); and a piezoelectric sensor (Signal Conditioned Accelerometer, Model 3145, Measurement Specialties, Inc., Fairfield, NJ), amplified by an EEG amplifier (P15D Preamplifier, Grass Quincy, MA). Cuff pressure was measured by a pressure transducer (Statham P23 XL) and a pressure processor amplifier (Gould, Valley View, OH) via a transducer protector (Gish Biomedical, Irvine, CA). Each subject's hand was placed on a wood board to hold the lower arm in place with all probes (Fig. 3). In order to determine the sensitivity of each of the pulse detection modalities, the pressure at which the pulse reappeared was calculated. This pressure was determined for each of the recordings for each modality (except for the Piezo sensor recordings from which no pulse wave signal was consistently obtained). The mean and standard deviation of the pressure at pulse reappearance was calculated for each of the 11 subjects. When a reappearing pressure of more than 2 of the 5 recordings could not be determined for a modality of one subject, data were discarded as being too small of a sample for the modality for that subject. Most recordings that were not used resulted from a poor signal-to-noise ratio, which was so

large that the pulse reappearance could not be determined. The pulse could not reappear at a pressure higher than the systolic pressure (Fig 5). These pressure values were averaged for each modality as shown in Table III.

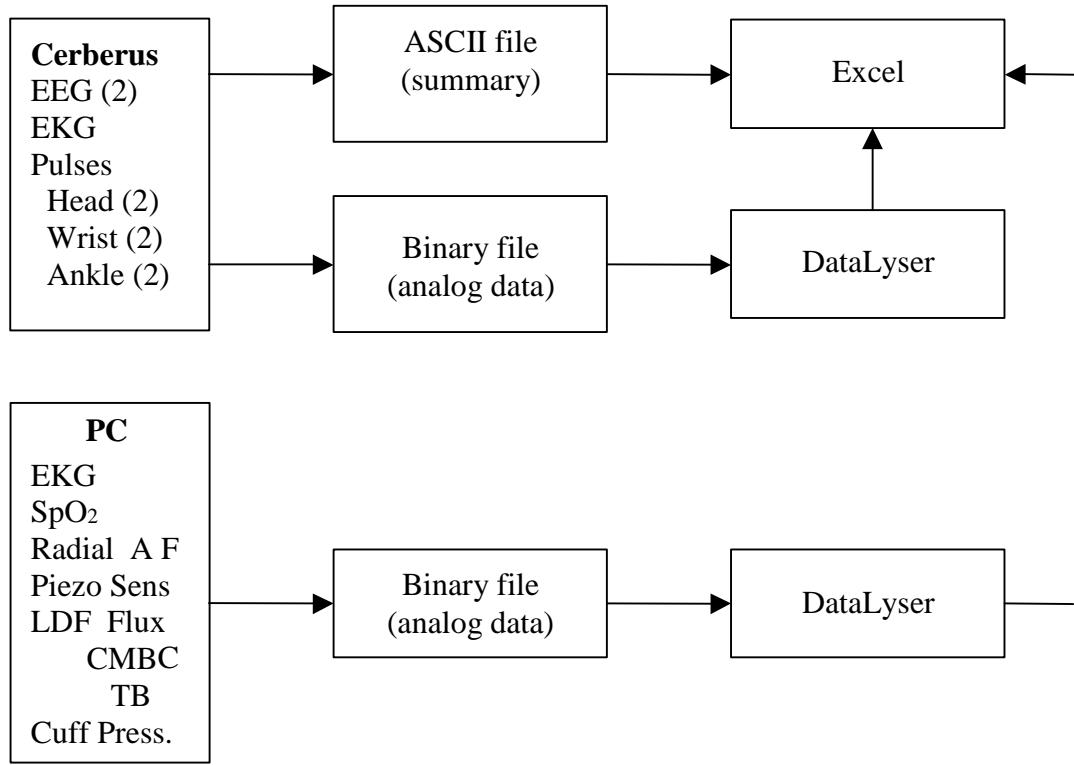


**Fig 3.** Sensor placement on arm and hand for pulse sensitivity measurement.

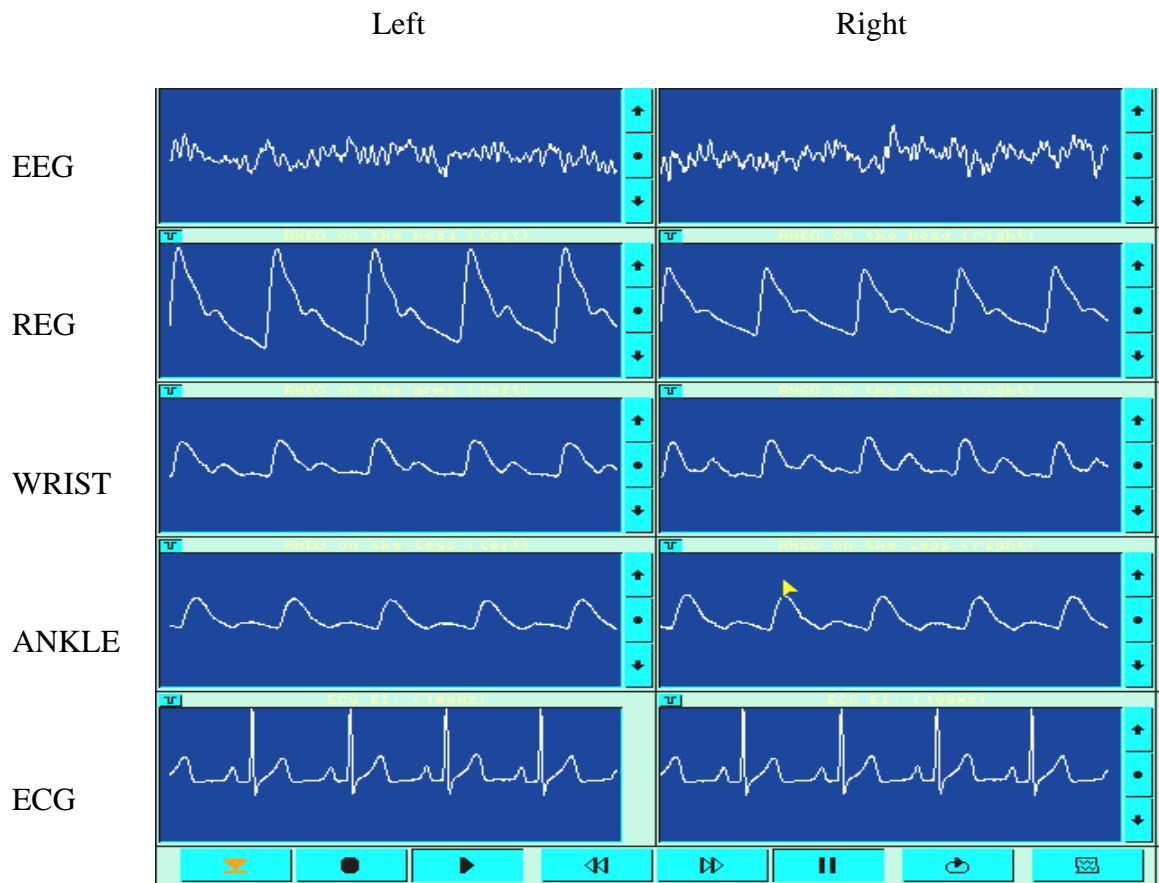
#### *Data Collection*

In each measurement, bioimpedance data were collected. Electrodes were placed on the head, arm and leg. ECG and EEG data were collected using the Cerberus system (A/D conversion 275 Hz; PCL-818 type A/D card, 12 bit resolution, Advantech, Sunnyvale, CA). Cerberus is discussed further elsewhere.<sup>25,26</sup> Data for the other pulse modalities were collected using the Datalyzer software (Baranyi, L) with another PC (A/D sampling rate 200 Hz, resolution 16 bits, PCI 6052E, National Instruments, Austin, TX) (Fig. 4). ECG measurements were made by both systems to assist pulse identification. The Cerberus system was used to average REG and peripheral pulse signals for each recording (pulse peak time, amplitude, and PWTT, Fig 1). In order to calculate pulse wave velocity (PWTT/distance), the distances were measured between the processus

xyphoideus and the points halfway between the two bio-impedance electrodes placed on the head, wrist, and ankle. Datalyzer software was used with cursor operation to generate pulse sensitivity data. Pulse detection sensitivity was expressed as the cuff pressure at which the pulse signal reappeared for each individual modality after blood flow was stopped.



**Fig 4.** Schematics of data collection (shown in three sections: left, center, and right). The sources of data (left) were the Cerberus system and a PC. In the Cerberus section, the number of analog channels used appear in parentheses. In the PC section, the modalities were as follows: ECG (electrocardiogram); SpO<sub>2</sub> (pulse oximeter); radial AF (radial artery flow); Piezo sens (piezoelectric sensor); and three LDF modalities --flux or flow, CMBC or concentration of moving red blood cells, and total backscatter or TB; cuff press (cuff pressure on the upper arm). In the ASCII file section (center), the values of signal averaging in Cerberus were derived from a 1-minute signal and were imported into an Excel file. PC signals were also 1-minute recordings; data extraction was performed using a cursor reading. The center section shows data format. The right section indicates the software used. Excel is a product of Microsoft, Redmond, WA; DataLyser, developed by L. Baranyi at WRAIR, was used for A/D conversion, visualization and quantification of presented analog physiological signals.



**Fig 5.** Cerberus traces (ECG is the same on both side)



**Fig 6.** Researchers in the Department of Resuscitative Medicine at WRAIR collect data for the present study.

#### *Statistical Analysis*

Blood pressure, heart rate, EEG and REG were measured as a potential source of biological variability and also for reference purposes. To measure variability, several methods were used, including variance, standard deviation, coefficient of variability (standard deviation/mean) for PWTT, pulse peak time, and amplitude of each conductive fabric at each peripheral site (both arms and legs). These coefficients of variability were averaged for each conductive fabric to obtain a general overview of the overall variability of each conductive fabric. Also, the arm and leg amplitudes of the bio-impedance pulse were averaged for each conductive fabric.

Intra and inter-variability were calculated as follows: 1) intra-individual variability (variance of each individual), and 2) mean of variances (inter-individual variability. step 1) mean of each individual, and step 2) Variance of means. Student t-tests were performed to determine the significance of the differences between the bioimpedance modality and the other pulse measurement modalities.  $P < 0.05$  was considered significant. Data are mean  $\pm$  SD.

## RESULTS

### *In vitro Down Selection*

The criteria for selecting conductive elastic fabrics as electrodes were as follows: optimal electrical resistance to the impedance amplifier; suitability of fabric for sewing into a uniform; no change in resistance as a function of extension; fully circumferential, being elastic. Using these criteria for the 13 pre-selected conductive materials, 8 were eliminated, and 5 were selected for *in vivo* testing (Table I). The original Cerberus electrode was the Rheoscreen, which is Velcro-based conductive fabric with silver covering layer, but non-elastic and the silver material was easily detached and is not commercially available. Before the start of *in vivo* studies, new antistatic groundings (All-Spec Industries, Inc., Wilmington, NC) were obtained and tested for applicability, then used in the study.

**Table I.** Specific conductivity of tested conductive fabrics and conductor wrist straps (personal antistatic groundings). The measuring tool was a digital multimeter (HD 100,

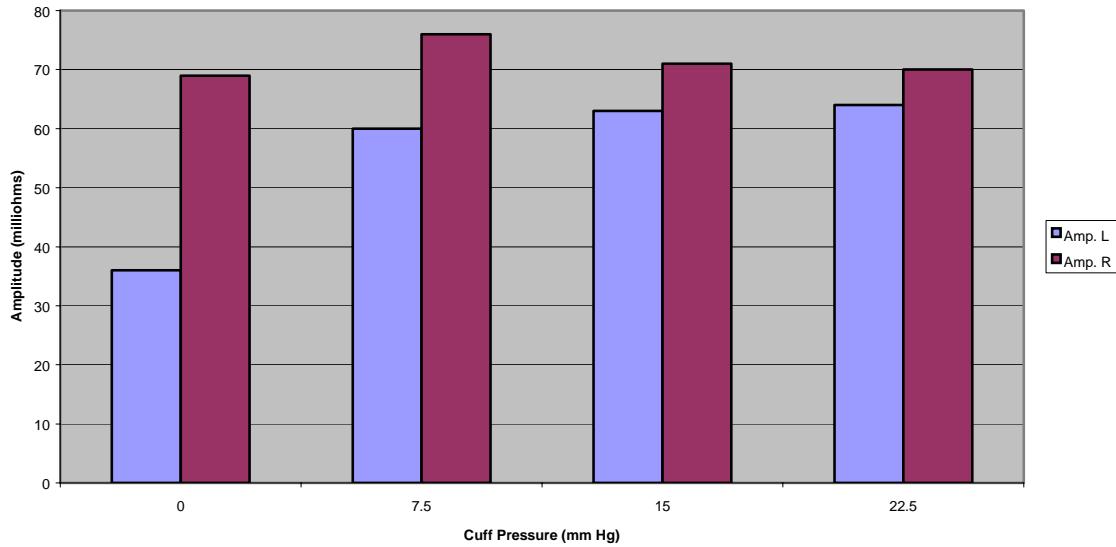
Material	Width	Conductive Surface (mm)	Elastic	Specific Conductivity Mean ± SD (ohm/cm)
	(mm)	(mm)		
Foster Miller Conductive F.	22	8	Yes	0.074 ± 0.027
Shielding Textile (Cu) #	17	17	No	0.501 ± 0.053
Velcro HI-MEG (Ag)	25	25	No	0.141 ± 0.030
Velcro Stainless Steel Hook	25	25	No	0.522 ± 0.094
Velcro Stainless Steel Loop	25	25	No	0.349 ± 0.032
Semitronics PK 2050	20	10	Yes	0.151 ± 0.077
Rheoscreen (Medis GmbH)	20	20	No	0.728 ± 0.461
SP1201*	19	14	Yes	2.568 ± 0.258
3M4720*	17/15	14/13	No	N/A
3M2368*	17	12	Yes	8.255 ± 0.967
Bam EA*	17	17	Yes	2.93 ± 2.202
S 7920*	19	15	Yes	39.06 ± 2.370
S 7933*	19	15	Yes	104.14 ± 14.740

Beckman, Fullerton, CA). For other details see Figure 1. Data are mean ± SD; n = 10 for each item. \* Conductor wrist straps (All-Spec Industries, Inc., Wilmington, NC); # Pannonshield Kft, Hungary)

#### *In vivo Down Selection*

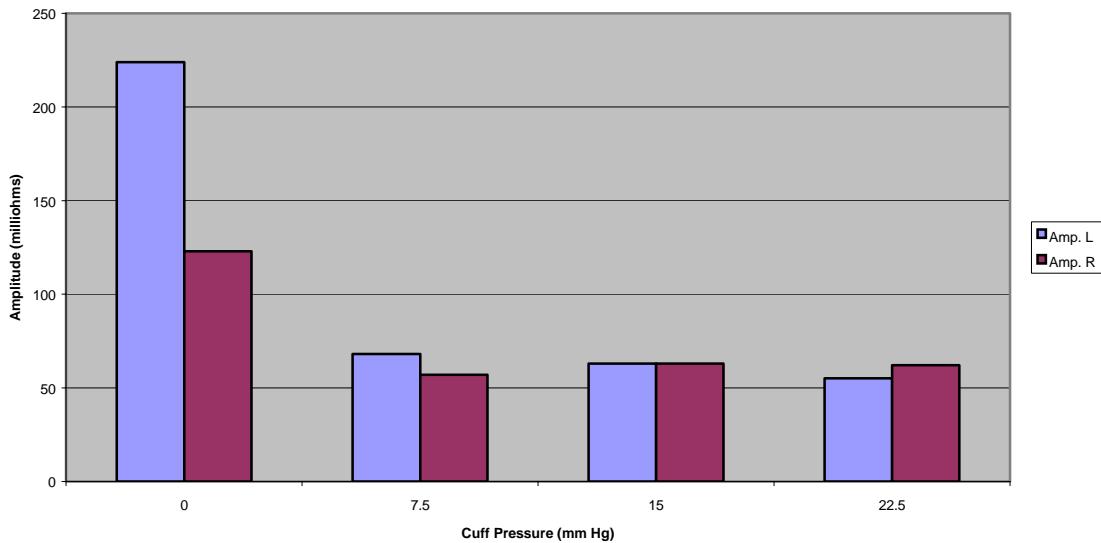
The results of the cuff pressure test were inconclusive, and no external pressure was applied in the later phases of the study.

**Arm Amplitude vs. Cuff Pressure**



**Fig 7.** Bio-impedance pulse amplitude on arm (above) and leg (below) during various cuff pressures. L: left side, R: right side. Mean of 1-minute recording. X units: milliohm.

**Leg Amplitude Vs. Cuff Pressure**



The down-selection criteria of the 5 conductive fabrics were as follows: ability to record pulse waves and ECG simultaneously and the presence of a high variability in the pulse amplitude measurement. Conductive fabrics were excluded from further selection for the following reasons: bad contact between conductive fabrics and 4 mm contact snap; no 4 mm contact snap; no full circumferential conductive fabrics; change in

resistance due to extension; incompatible resistance to the impedance amplifier; inappropriate conductivity (deregulating ECG amplifier in Cerberus system). These criteria were used to reduce the 5 conductive fabrics to 2. The Hi-Meg conductive fabric is no longer in production and was therefore not among the two conductive fabrics chosen for use in the next phase of the study. The TM2204 and SP776 conductive fabrics were chosen for use in the measurements made in the next phase. This determination was made because the TM2204 and SP776 conductive fabrics have better reproducibility (less variability in the signal) than the SP7933 and TM2272 conductive fabrics. The amplitude advantages of the two selected conductive fabrics over the other fabrics were insignificant compared to the loss of reproducibility with these two conductive fabrics (Table II).

Electrode	Amplitude (milliohm)		Coefficient of Variability
	Arm	Leg	
1. Hi-Meg	$176 \pm 114.56$	$681 \pm 28.58$	$0.17 \pm 0.21$
2 .SP 7933	$168 \pm 86.93$	$145 \pm 156.09$	$0.25 \pm 0.23$
3. TM 2272	$108 \pm 91.02$	$116 \pm 110.28$	$0.28 \pm 0.31$
4 .TM 2204	$77 \pm 25.66$	$73 \pm 41.20$	$0.17 \pm 0.17$
5. SP 776	$89 \pm 43.95$	$190 \pm 121.10$	$0.16 \pm 0.24$

**Table II.** Amplitude of bio-impedance signal and coefficient of variability of five conductive fabrics. The Hi-Meg conductive fabric had the largest mean amplitudes for both arms and legs; however, extreme high amplitude occurred on the leg (681 mohm), resulting in overshoot (no value).

The coefficient of variability (standard deviation/mean) was calculated for the pulse wave transmission time, peak time, and amplitude of each conductive fabric at each peripheral site (both arms and legs). These coefficients of variability were averaged for each conductive fabric to obtain a general overview of the overall variability of each conductive fabric. Also, the arm and leg amplitudes were averaged for each conductive fabric.

Electrodes 1, 4, and 5 were found to have the smallest average coefficient of variability. Electrode 5 was chosen as one of the two electrode sets to be used in the next phase because of its ability to reproduce pulse wave times with minimal variance. Electrode 4 was chosen over Electrode 1 because Electrode 4 uses an elastic band instead of Velcro, which made it a more practical choice for sewing into a uniform. N = 2 subjects, 10 measurements for each conductive fabric. Data are mean  $\pm$  SD.



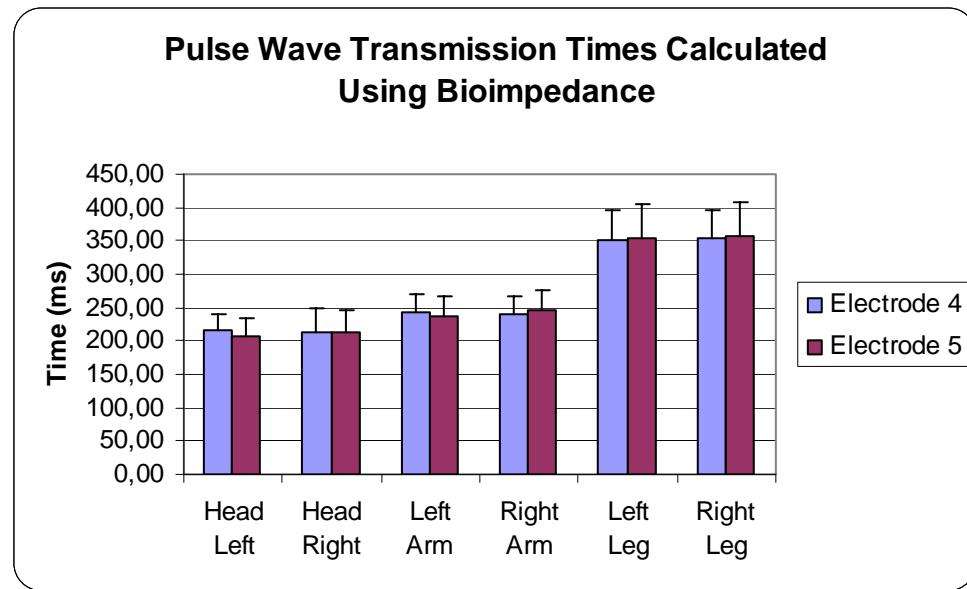
**Fig 8.** TM2204 (Electrode 4)  
Fabric Wrist Strap Band  
3M, US Pat: 4,720,765



**Fig 9.** SP776 (Electrode 5) Velcro  
Adjustable Red Wrist Strap (GRD-TRONICS)

### *Variability of Physiological Signals*

Systolic, diastolic, mean blood pressure, heart rate, EEG, and REG were measured and compared to peripheral bio-impedance pulses. The variability of bio-impedance signals was in the same range as blood pressure, heart rate and EEG. The intra-individual variability of the EEG frequency was higher than its inter-individual variability; a similar relationship was observed for the EEG power (Table III, A,B,C).



**Fig 10.** Pulse wave transmission times.

A	Intra-Individual Variance		Inter-Individual Variance	
	Mean of Variances	SD	Variance of Means	SD
Heart Rate	9.30	6.85	59.36	8.51
Systolic BP	24.96	18.10	126.53	18.06
Diastolic BP	12.14	11.23	68.52	7.14
Mean BP	6.70	6.19	89.40	6.48

B	Intra-variability		Inter-variability	
	C O V	SD	C O V	SD
SAP	0.83	0.11	0.12	0.03
PWV				
TM2204	1.87	0.73	0.13	0.03
PWV				
SP776	1.17	0.31	0.14	0.01
EEG F	1.10	0.10	0.52	0.02
EEG P	1.64	0.56	0.03	0.00

C	Pulse Wave Velocity			
	TM2204		SP776	
	Mean (cm/s)	SD	Mean (cm/s)	SD
Head Left	202.67	24.03	214.11	31.18
Head Right	204.95	31.87	210.08	35.69
Left Arm	305.22	33.43	319.15	45.32
Right Arm	306.39	33.39	310.58	46.40
Left Leg	328.11	54.05	324.90	54.14
Right Leg	322.47	48.57	321.57	45.90

D	Physiological Modality	C O V
Pressure of Pulse Reappearance	Pulse Oximeter	0.17
	Ultrasound Doppler	0.07
	Laser Doppler	0.09
	Bioimpedance (TM2204)	0.05
	Bioimpedance (SP776)	0.07
Blood Pressure Measurement	Heart Rate	0.15
	Systolic BP	0.15
	Diastolic BP	0.11
	Mean BP	0.08

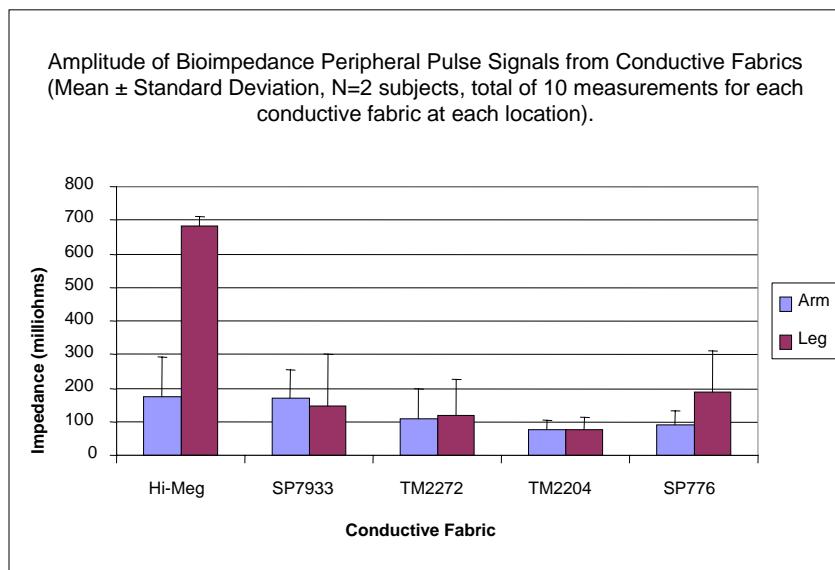
**Table III.** Comparative results of pulse waves and other physiological modalities on their variability during resting condition. Legend: (A, B, and C) and during the pulse sensitivity test (D). Standard Deviation: SD., BP: blood pressure.

A. Variability of blood pressure and its derivatives. HR (heart rate); systolic blood pressure; diastolic blood pressure; and mean blood pressure. N = 11 subjects, 110 trials.

B. Intra- and inter variabilities were made for each measurement, and the standard deviation was found so that the coefficient of variability (COV) could be calculated. These CV's were then averaged for each measurement group (i.e. SAP), and the SD of the COVs was calculated. Data are n = 10 subjects, 50 trials for each electrode, 100 trials for EEG.

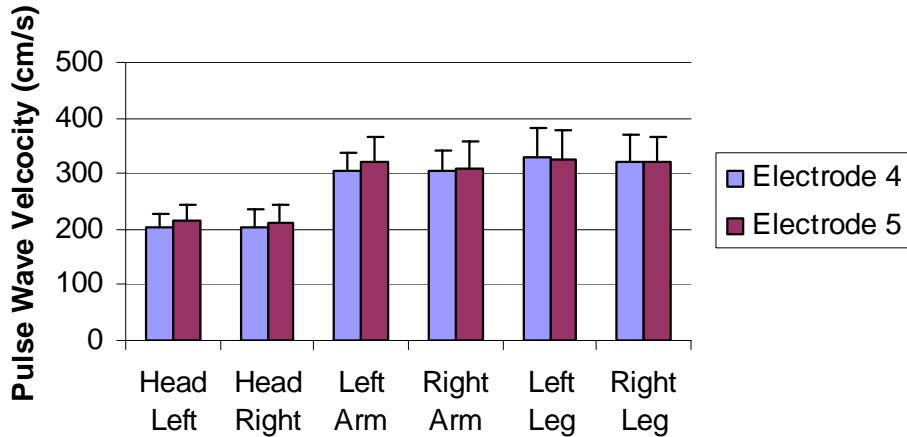
C. Pulse wave velocities from bio-impedance using the two best conductive fabrics (n = 11 subjects, 50 trials with each conducting fabric).

D. Coefficient of variability of pulse waves and blood pressure derived data during pulse sensitivity test. Coefficient of variability was calculated from pressure of pulse reappearance of each pulse modality. Student t-tests determined that the differences between the bio-impedance modality using TM2204 and SP776 and the two Doppler modalities were insignificant ( $P > 0.05$ ). Also, the differences between the sensitivities of the bio-impedance modality using TM2204 and SP776 were also determined to be insignificant. Data are n = 10 subjects, 50 trials for each electrode, 100 trials for other modalities.



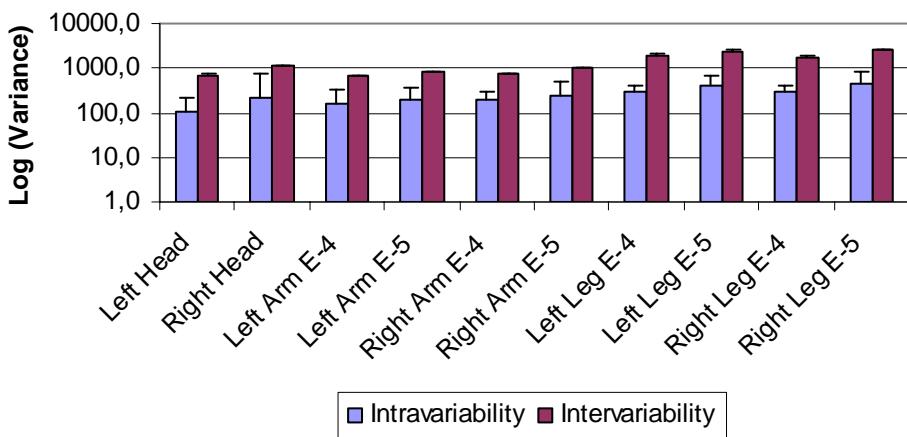
**Fig 11.** Amplitude of the bio-impedance signals for each of the conductive fabrics. The amplitude of the pulse signal for each conductive fabric was plotted to determine which had the largest amplitude. The Hi-Meg conductive fabric had the largest mean amplitudes for both the arms and the legs.

### Pulse Wave Velocities Calculated Using Bioimpedance



**Fig 12.** Pulse wave velocity data for Electrode 4 and 5, showing the same variability

### Inter- and Intra-Variabilities of Bioimpedance



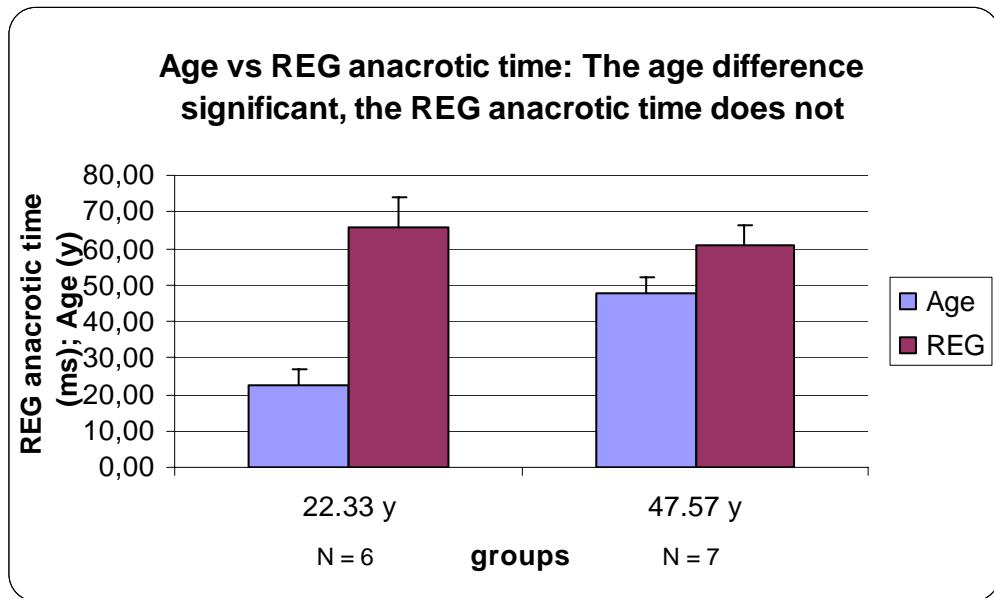
**Fig 13.** Intra individual and inter individual variability of pulse wave velocity for Electrode 4 and 5.

## *REG and EEG*

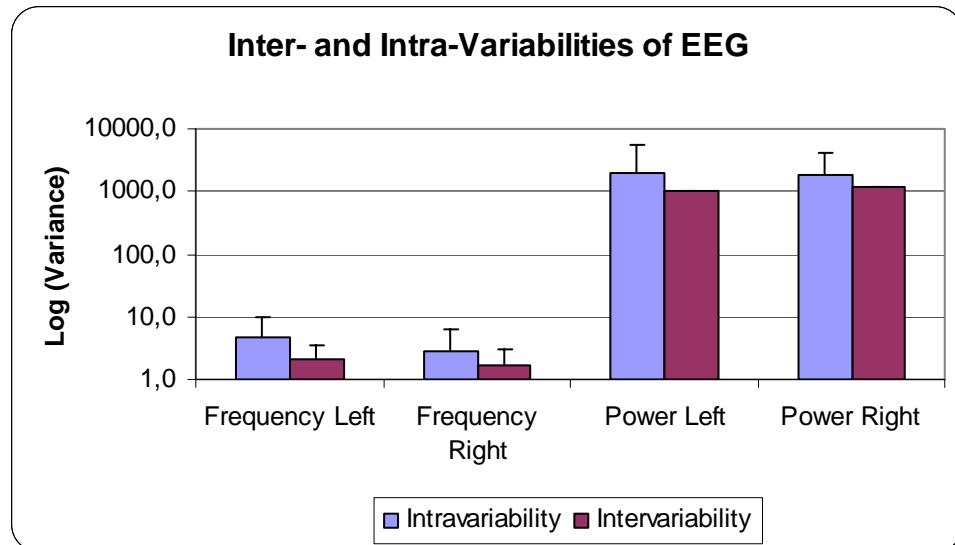
For all healthy subjects, physiological cerebrovascular aging was expected to show an identical slope with age. The REG anacrotic time showed a nearly identical slope for both REG (1.79) and age (3.45; ratio: 0.52) (Fig. 5).

	young		old	
	Age year	REG ms	Age year	REG ms
Mean	22,33	65,50	47,57	60,71
SD	4,37	8,34	4,58	5,65
Count	6	6	7	7
P			>0,0001*	0,0001*

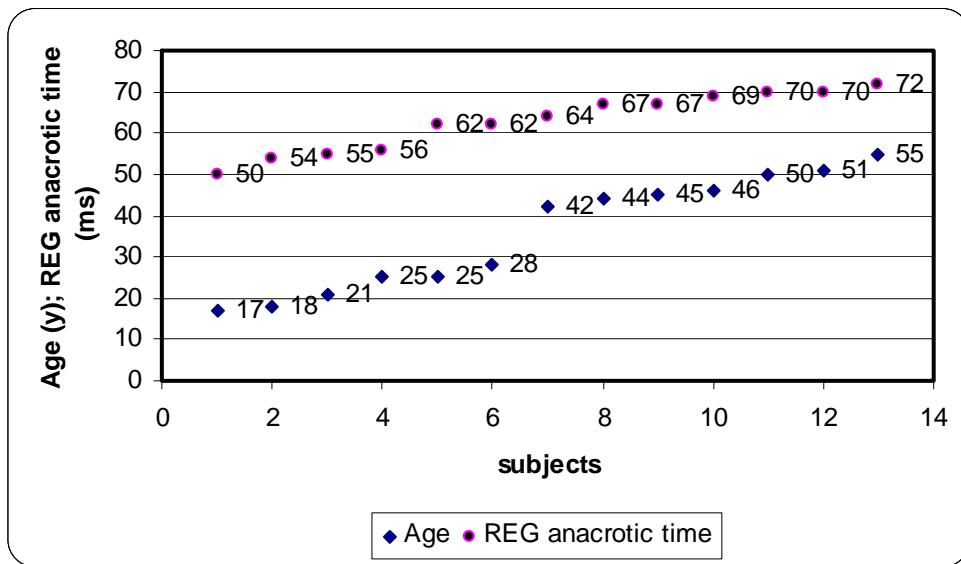
**Table IV.** Numeric characteristics of REG anacrotic time and age. REG samples were analyzed dividing into subgroups according to age: young (17-28) and old (42-55). \* P values of difference between young and old group.



**Fig 14.** Age and REG anacrotic time.



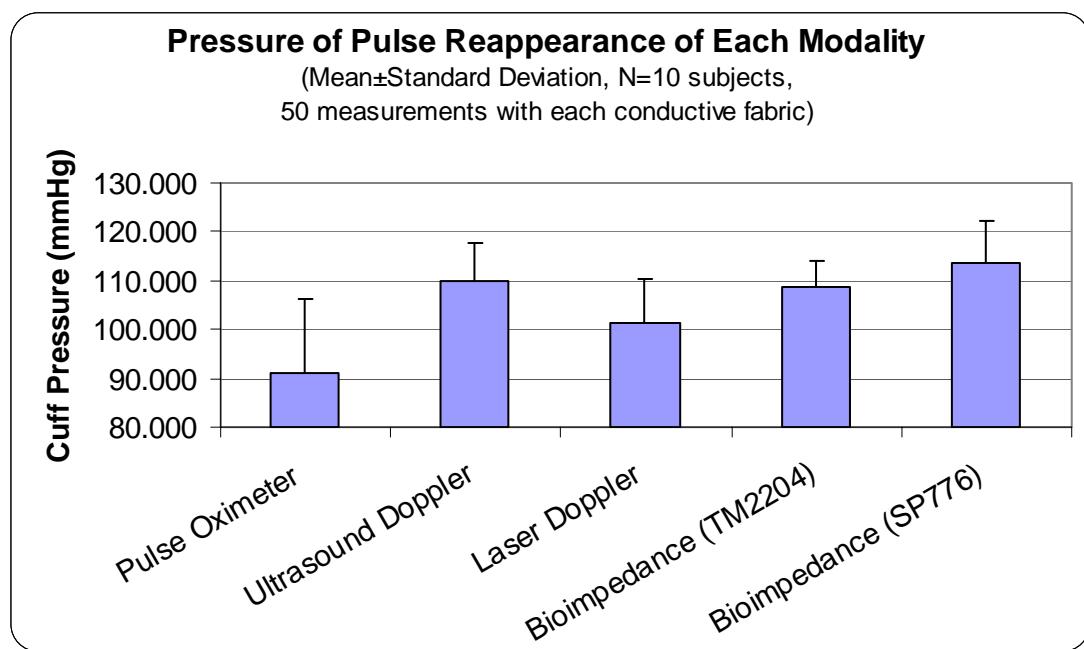
**Fig 15.** Intra individual and inter individual variability of dominant frequency of EEG power spectrum.



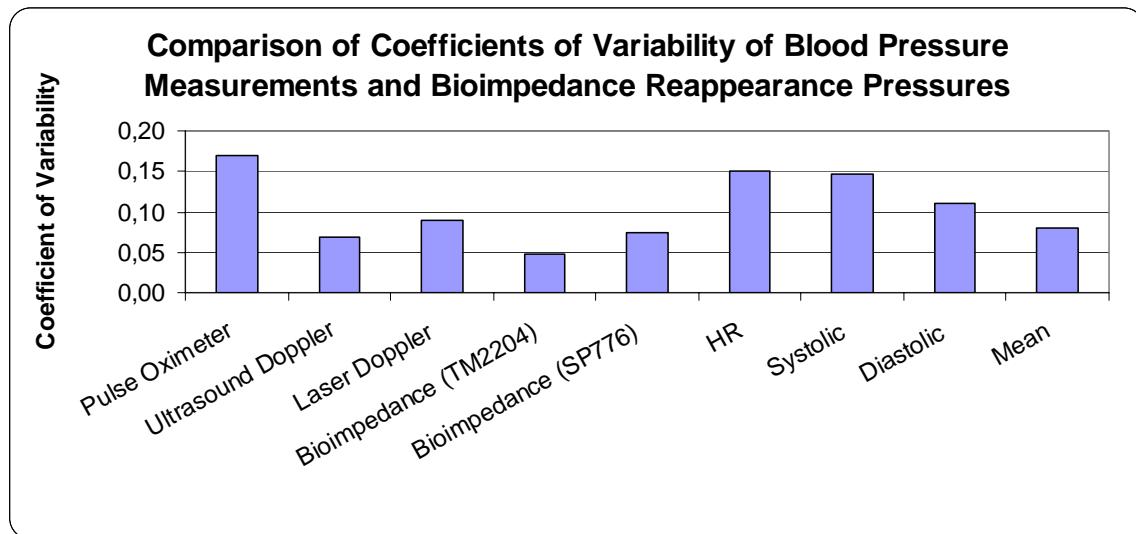
**Fig 16.** Sorted age and REG. Age and REG regression data (calculated as one group, n=13) are as follow: Age:  $y = 3.4505x + 11.769$ ,  $R^2 = 0.9509$ , and REG:  $1.7912x + 50.385$ ,  $R^2 = 0.9527$  (both linear).

### *Pulse Sensitivity*

Pulse waves were measured during arm cuff inflation and deflation (Figure 5). The applied pulse sensitivity test resulted in no significant differences among the pulses of the pulse oximeter, laser Doppler, Doppler ultrasound, and the bio-impedance measurement technique (Table III, D). When measuring with either of the two conductive fabrics, the bio-impedance modality showed no statistically significant differences with the laser Doppler and ultrasound Doppler modalities. Also, for each conductive fabric, the difference between the bio-impedance modality and the pulse oximeter modality was determined to be statistically significant with the bio-impedance method having the greater sensitivity. T-tests comparing the bio-impedance modality using TM2204 and the bio-impedance modality using SP776 showed no statistical significance in the differences (Figure 9, below).

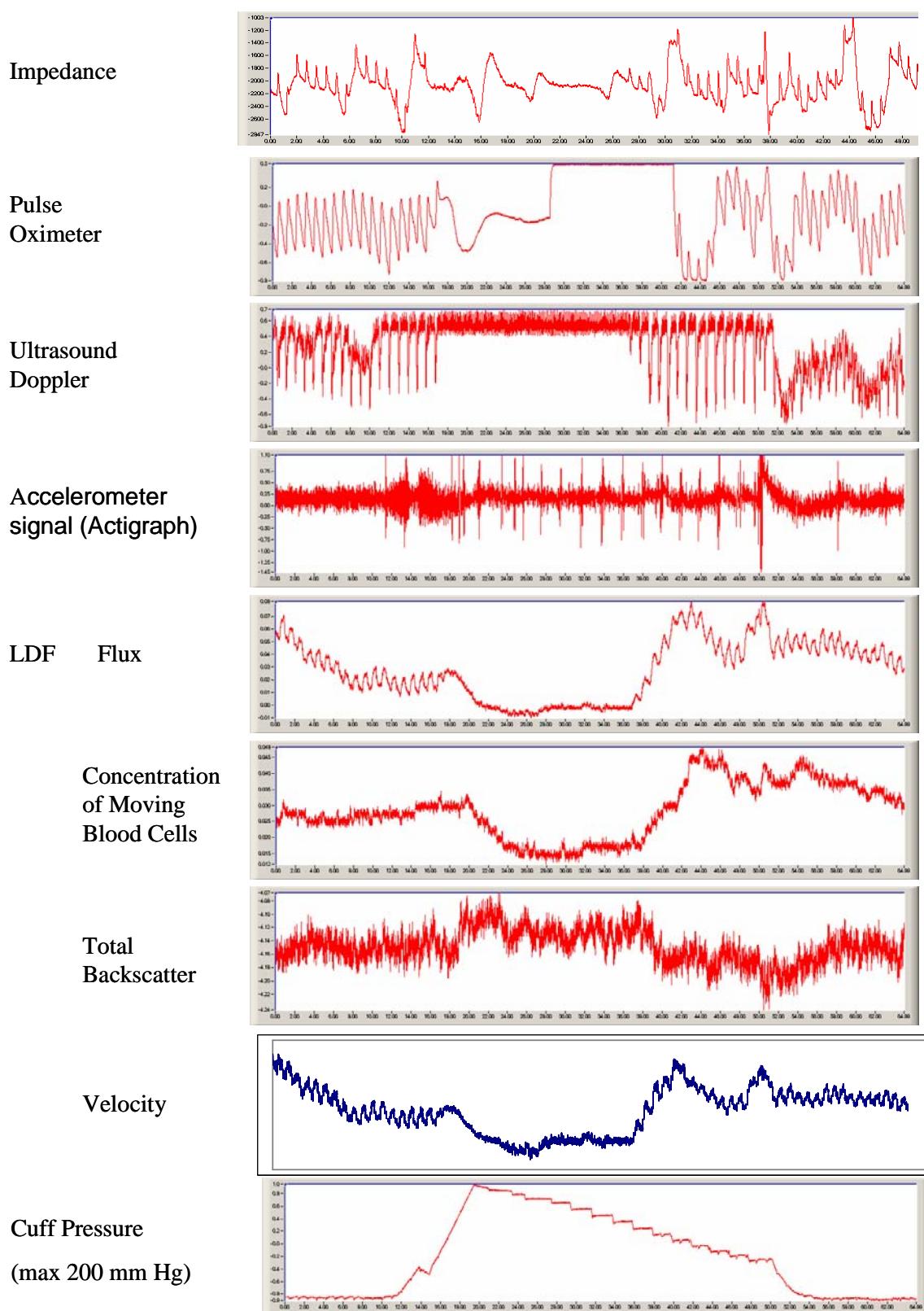


**Fig 17.** Arm cuff pressure of pulse reappearance of each modality.



**Fig 18.** Coefficients of variability (COV) mean divided by the standard deviation) of blood pressure measurement (HR: heart rate, systolic, diastolic and mean blood pressure), three pulse modalities, and bioimpedance reappearance pressures in case of two electrodes. Data groups involved 13 subjects. Using COV made possible to compare different modalities and units.

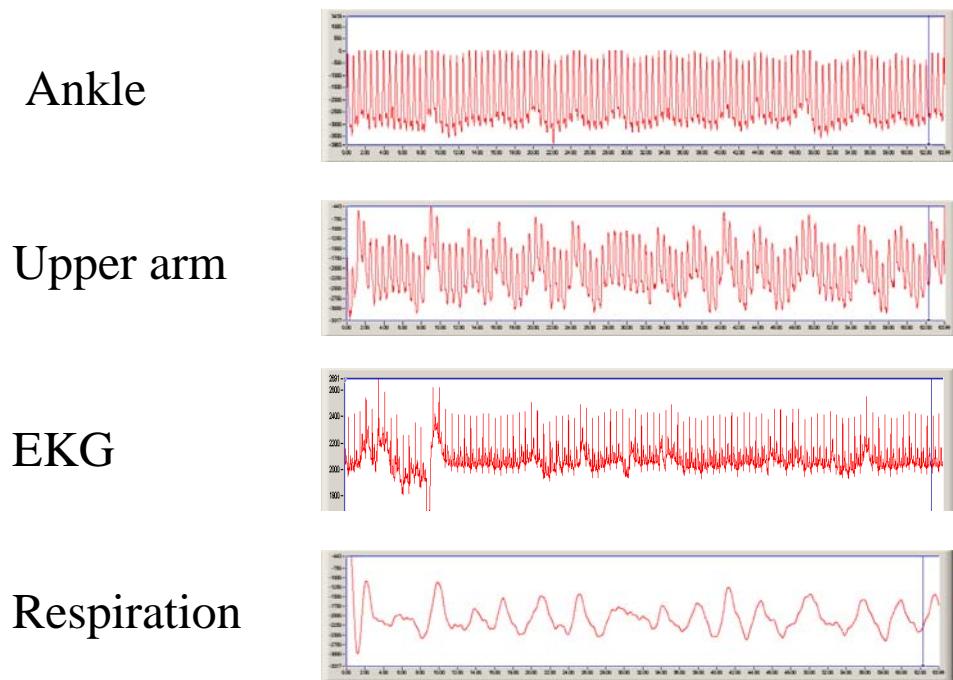
The sensitivity of the bio-impedance measurement was comparable to the sensitivities of the pulse oximeter, laser Doppler, and ultrasound Doppler. There was no statistical difference between the bi-impedance and the ultrasound Doppler and laser Doppler measurements. Recordings made with the SP776 conductive fabric had a higher average pressure for pulse reappearance than recordings with the TM2204; however, the difference was not statistically significant. In general, the signals recorded using SP776 were less noisy than signals recorded using TM2204, and thus, SP776 produced more usable data. After the completion of this study, bio-impedance remains a potential method for monitoring heart rate, PWV of the soldiers.



**Fig 19.** Tracings of pulse sensitivity measurement. The left part was used as a control before arm cuff inflation, indicated on the lower trace. During cuff pressure elevation, all pulse waves disappeared. Ultrasound polarity is up side down. LDF velocity was calculated as flux/concentration of moving blood cells. Different amplifiers have different time constant which influenced the cessation and recovery of the given signal. As an illustration of this statement, see the pulse oximeter signal. Time window was 1 minute, subject # H-3.

#### *Technical novelty*

Cerberus hardware and software produced a 2-channel bio-impedance signal or pulse wave, and ECG.



**Fig 20.** Two channel bio-impedance recording (1 minute) from upper arm and ankle – as original signals. ECG generated by Cerberus hardware without designated electrode and Respiration generated by software extraction (DataLyser) from Upper arm signal.

## DISCUSSION

Here we report results of a descriptive study involving three components: electrode down-selection; comparison of variability of bio-impedance and other physiological signals; pulse sensitivity analysis. There was no statistical difference between the bio-impedance measurement and the other techniques. Potential bio-impedance vital sign monitor hardware and software were used to perform these measurements. The specific advantage of using only bio-impedance electrodes is that this method produced a 2-channel bio-impedance signal and an ECG and respiratory trace can also be generated from the signal. Two of the conductive fabrics tested as peripheral electrodes were effective: SP776 and TM2204. The two fabrics were equally sensitive, but SP776 showed better signal-to-noise ratio and a better EGG signal than TM2204. Related validation studies using bio-impedance and detailing several aspects of applications of bio-impedance were reported and discussed earlier.<sup>10-13</sup>

### *Monitoring*

Biosignals can be divided into two main groups: deterministic and stochastic signals. Their characteristics were detailed elsewhere; here we note only that the variability of deterministic and periodic signals, such as ECG, blood pressure and bioimpedance, were in the same range as variability of EEG –derived variables. EEG, however, is a stochastic signal.<sup>28</sup> One possible explanation is the applied signal/noise ratio improving procedure, “the signal averaging” used in the Cerberus system.

Life sign monitoring is important for saving lives and has both military and civilian medical applications as well as usefulness for aviation and space research. Ideally, life sign monitoring devices would be capable of collecting physiological signals *continuously* and *non-invasively*.<sup>29,30</sup> Combat casualty care has additional and particular requirements beyond those required under hospital or laboratory conditions.<sup>1,2</sup> Multifunctional electrodes are desirable for life sign monitoring in both cases but are particularly valuable for combat casualty, aviation, and space environments.

Since the dominant cause of death in combat is hemorrhage, one of the main vital signs is blood pressure. The cuff technique is not ideal for triage. Recently available noninvasive blood pressure devices measure blood pressure on the hand or lower arm, typically requiring positioning the sensor above the radial artery. This makes them far from optimal in the military environment. PWTT is a potential signal to estimate noninvasive blood pressure using bioimpedance which can be realized with wearable electrodes. Recent results support this potential; however, further studies are needed to eliminate error caused by low blood pressure values. In the future we will test the application of other physiological signals as well, since the potency and maximum feedback gain of various arterial pressure control mechanisms (baroreceptor, chemoreceptor and central nervous system ischemia) are different;<sup>31</sup> the brain, which is the most sensitive sensor to hypoxia and ischemia, seems to be the optimal organ for use as a feedback modality for life sign monitoring and resuscitation; consequently we performed studies testing biomedance monitoring on the head.<sup>32-37</sup> Additional studies will be conducted to determine the sequence of cessation of vital signs, in order to apply this knowledge in future vital sign monitors used in the WPSM and LSTAT.<sup>24</sup>

### *Conclusion*

The ultimate goal of this research and development is to develop a palmtop, computer-based non-invasive, cuffless vital sign monitor for military (WPSM), aerospace and emergency medicine, as well as for neurosurgical, intra-operative and postoperative patient monitoring. The available related products do not use wearable electrodes.<sup>38,39</sup> In this study, we determined the variability and sensitivity of bio-impedance pulse measurement and compared it to other physiological signals.

For field applicability of the bioimpedance technique, further human and animal studies are needed to test the following: 1) manipulation of systemic arterial pressure involving pathological range of regulation; 2) tests of additional conductive fabrics; 3) construction of a prototype of a bio-impedance-based vital sign monitor using the tested hardware and software.

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